## REMARKS

### Status of the Claims

Claims 1-12, 19 and 20 are currently pending in the application. Claims 1-6, 9-12 and 19 stand rejected. Claims 7 and 8 are allowed. Claims 1, 4, 9 and 19 have been amended herein. All cancellations and amendments are made without prejudice or disclaimer. New claim 20 has been added herein. No new matter has been added. Support for claim 20 may be found in the specification at page 17 to page 19 and Figures 6 and 8. Reconsideration is respectfully requested.

Furthermore, Applicants again request the Examiner to record or note the change of address regarding the Power of Attorney for this application (see also the original Revocation of Power of Attorney of October 7, 2002, copy attached to Amendment of June 28, 2005).

### Rejections Under 35 U.S.C. § 102(e)

Claims 9 and 10 stand rejected under 35 U.S.C. § 102(e) as assertedly being anticipated by U.S. Patent No. 5,928,914 to Leboulch et al. (hereinafter referred to as "Leboulch et al."). (*See*, Office Action of September 22, 2005, at page 3, hereinafter referred to as "Office Action"). Applicants traverse the rejection as set forth herein.

Specifically, it was thought that Leboulch et al. disclose a vector comprising two *loxP* sites that cannot be recombined. However, Leboulch et al. do not disclose each and every element of claim 9. Claim 9 recites, in part, that the mutant *loxP* sequence comprises a sequence of which a part of said inverted repeat sequence 1 of *loxP* is mutated, emphasis added.

The mutant loxP disclosed by Leboulch et al. is similar to that of the present invention only in the respect that the two types of loxP are obtained by introducing point mutations into wild-type loxP. However, from that singly shared concept, the two inventions diverge and differ dramatically in structure and mechanism of action. The differences pertain not only to the site at which the mutation is introduced, but also in terms of the basic mechanism for improving the recombination efficiency of genetic elements as a result of the placement of the mutation.

The loxP sequence is divided into three (3) basic structures: inverted repeat sequence 1, a spacer sequence, and inverted repeat sequence 2, in sequential order. Of these, it is understood that the inverted repeat sequences 1 and 2 are sequences recognized by the Cre recombinant enzyme, and that the spacer sequence is a sequence in which a recombination reaction actually takes place. (*See*, Supplemental Figure 1, attached hereto as Exhibit A).

The mutant loxP of the disclosure of Leboulch et al. is characterized in that, among the three regions, it has a mutation in the spacer sequence. (*See*, Leboulch et al., for instance, at column 5, lines 43-51, wherein it is stated that "the other incompatible lox sequence can be a mutated form of the LoxP1 sequence, for example, having a point mutation *in the eight nucleotide spacer sequence*," emphasis added, and further at lines 18-25, wherein it is stated that incompatibility can be achieved by preferably mutating "one of two identical lox sequences, preferably in their spacer sequences," also see, for instance, claim 3 reciting a mutation in the spacer sequences). Because of the Leboulch et al. mutation, when wild-type loxP and mutant loxP exist in a single vector, a side reaction such as recombination between the two types of loxP does not occur, and as a result, the recombination efficiency of genetic elements of interest is

improved. However, it is also true that the reverse reaction, whereby a product of interest returns to a pre-reaction state, may, and does, occur.

In contrast, the mutant loxP of the present invention has a mutation in the inverted repeat sequence 1 or 2. Because of such mutation, when wild-type loxP and mutant loxP exist in a single vector, as with the aforementioned example, although recombination between the two types of loxP cannot be completely prevented, a reverse reaction whereby a product of interest returns to a pre-reaction state is *strongly decreased.* (*See*, especially, Specification, at page 13, lines 8-21). Thus, the recombination efficiency of genetic elements of interest is substantially improved by the present invention. (*See*, Supplementary Figure 2, attached hereto as Exhibit B). To make this point more clear, claim 1 has been amended to recite, in part, "wherein the mutant *loxP* sequence comprises a sequence in which a part of said inverted repeat sequence 1 of *loxP* is mutated such that recombination of the mutant *loxP* occurs more efficiently than the reverse reaction as compared to wild-type *loxP*."

Thus, not only are the structures of the loxP disclosed by the present invention and that disclosed by Leboulch et al., completely different, but so too is the mechanism of the mutant loxP of the present invention also different from that disclosed by Leboulch et al., with regard to the improvement of the recombination efficiency of genetic elements. For example, lox2272 is a mutant obtained from lox511, which is one of the mutants disclosed and used in Leboulch et al. Both lox2272 and lox511 have mutations in their spacer regions, and thus it is anticipated that recombination can be efficiently carried out by a single mechanism. However, lox66 and lox71 of the present invention have mutations in their inverted repeat sequences. When these mutants

are used in a recombination reaction, an unexpected and heretofor unobserved increase in recombination efficiency is achieved. (*See*, Exhibit B).

Leboulch et al. disclose a recombination reaction which occurs in the sequence portion of loxP, namely, in the spacer sequence thereof. In contrast, the present invention uses this same sequence to change the recombination efficiency. Accordingly, all types of mutant loxP contained in the vectors disclosed by Leboulch et al. have mutations in their spacer sequences. (*See*, Leboulch et al. at column 5 and claims 1-5).

In contrast, the present invention discloses a construct in which the spacer sequence of loxP is NOT mutated. Rather, the sequence portions recognized by the Cre recombinant enzyme, namely, the inverted repeat sequence 1 or 2, are mutated. These portions are used to change the recombination efficiency. In particular, in claim 9 of the present invention, the mutant loxP described in (a) has a mutation in the inverted repeat sequence 1, whereas the mutant loxP described in (b) has a mutation in the inverted repeat sequence 2. Thus, the present invention discloses two types of loxP described in (a) and (b) in combination to generate a new mutant loxP having mutations in both the inverted repeat sequences 1 and 2, thereby causing an unexpectedly marked change in recombination efficiency.

Thus, Leboulch et al. do not disclose each and every element of the present invention because, as shown above, the structures of the two constructs are different and, their mechanism of action are also different. Furthermore, Leboulch et al. could not possibly be interpreted to disclose the present invention because Leboulch et al. do not achieve the same magnitude of recombination efficiency as achieved through the present invention. Therefore, Leboulch et al. does not anticipate the presently claimed invention, as claimed in claim 9.

Dependent claim 10 is not anticipated as, *inter alia*, depending from a non-anticipated base claim, claim 9.

Reconsideration and withdrawal of the anticipation rejection of claims 9 and 10 are respectfully requested.

**Rejections Under 35 U.S.C. § 103(a)**

Claims 1-6, 11 and 12 stand rejected under 35 U.S.C. § 103(a) as assertedly being unpatentable over Leboulch et al. in view of Araki et al., *Targeted Integration of DNA Using Mutant Lox Sites in Embryonic Stem Cells*, *Nuc. Acids Res.*, 1997, 25(4):868-872, hereinafter referred to as "Araki et al." (Office Action, at page 3). Applicants traverse the rejection as hereinafter set forth.

M.P.E.P. § 706.02(j) provides the standard for establishing a *prima facie* case of obviousness as follows:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Specifically, the Examiner states that Leboulch et al. also teach that one of the two *loxP* sequences can be a *loxP1* and a mutant form of *loxP* and that Araki et al. teach site directed integration using *lox71* and *lox66* in mouse ES cells. (*Id.* at pages 3-4).

Regarding Leboulch et al., Applicants refer the Examiner to the prior discussion concerning the identity of the sequences recited in the claims of the present application, that is, that Leboulch et al. only disclose mutations within the spacer region, not the inverted repeat region, of *loxP*.

Furthermore, Araki et al. only teach that recombination efficiency is increased by the use of *lox71* and *lox66* together, not a single vector containing both *loxP* and *lox71* OR *lox66*. Thus, Leboulch et al. even in combination with Araki et al. do not teach all the elements as claimed in claims 1 and 4 of the present invention. Therefore, Leboulch et al. and Araki et al. are not sufficient to support a *prima facie* obviousness rejection.

Additionally, the Examiner states that Araki et al. teach that *loxP* and *lox71* or *lox66* do not recombine efficiently in mouse ES cells. (*Id.*). However, Araki et al. contains no reference or data measuring the reverse reaction that yields the starting products. Araki et al. do disclose that "(t)he targeting frequencies for normal *loxP* sites were very low." (*See*, Araki et al. at page 870). The frequencies reported by Araki et al. in Table 1 are obtained using the final amount of a product of interest. This final result or yield of product includes any reduction of the amount of the product due to a reverse reaction, yielding the initial reactants. Thus, since the targeting frequencies were "very low," the recombination efficiency of the reverse reaction, yielding the initial starting material, must be rather high. In contrast, the invention of the present application is directed to mutations in two *loxP* inverted repeat sites that, when combined with *loxP*, create very low occurrence of the reverse reaction. Neither Araki et al. nor Leboulch et al. disclose this concept, or benefit therefrom.

Furthermore, although Applicants do not agree that the present invention is obvious in light of the references cited alone, or in combination as stated by the Examiner, to expedite prosecution, claims 1 and 4 have been amended to more clearly describe the present invention and recite, in part, "recombination of the mutant *loxP* occurs more efficiently than the reverse reaction catalyzed by a recombinase enzyme as compared to wild-type *loxP*."

Dependent claims 2, 3, 5, 6, 11 and 12 are non-obvious for similar reasons as discussed above.

Reconsideration and withdrawal of the obviousness rejection of claims 1-6, 11 and 12 are respectfully requested.

**Rejections Under 35 U.S.C. § 112, First Paragraph**

Enablement

Claim 19 stands rejected under 35 U.S.C. § 112, first paragraph, for assertedly failing to comply with the enablement requirement. (*See*, Office Action, at page 5). Applicants traverse the rejection as set forth herein.

Specifically, the Examiner states that critical components such as SA, IRES and a marker gene are required for the practice of the gene trapping method and that the disclosure does not enable a gene trapping method using vectors that comprise only *loxP* sites. (*Id.*). However, claim 7 has been allowed and recites a trap vector comprising only a marker gene. (*See*, claim 7(e)). Thus, claims directed to a trap vector comprising a marker gene, as in claim 7, must be enabled. Therefore, amended claim 19 is directed to a method of gene trapping, comprising introducing into an embryonic stem cell a trap vector comprising a *loxP* sequence, a marker gene

and a mutant *loxP* sequence. Claim 19, as amended, now recites the element "a marker gene," as suggested by the Examiner, and is now believed to be fully enabled by the disclosure. (*See*, Office Action, at page 5, wherein it is stated, "critical components such as SA, IRES and marker is required for the practice of the 'gene trapping method.'"). Support for this amendment can be found, for instance, in the as-filed specification at page 14 and in the original claims, such as claim 7.

New claim 20 has been added and is dependent from claim 19. Support for new claim 20 may be found in the as-filed specification, for instance, at page 14 and original claim 7.

Reconsideration and withdrawal of the enablement rejection of claim 19 is respectfully requested.

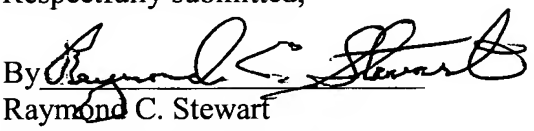
## CONCLUSION

If the Examiner has any questions or comments, please contact Thomas J. Siepmann, Ph.D., Registration No 57,374 at the offices of Birch, Stewart, Kolasch & Birch, LLP.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to our Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under § 1.17; particularly, extension of time fees.

Dated: March 22, 2006

Respectfully submitted,

By 

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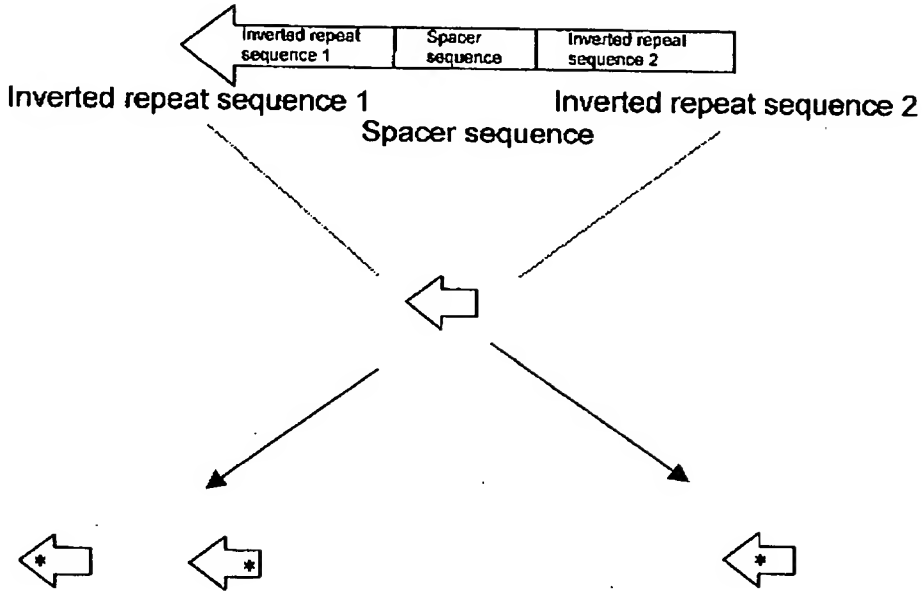
Attachments: Exhibit A and B

Supplemental Figure 1

## Structure of LoxP




Mutant loxP used in **the present invention** (having mutation in inverted repeat sequence thereof)

Mutant loxP used in Leboulch et al. (having mutation in spacer sequence thereof)

tabbies EXHIBIT A

Supplemental Figure 2